Frontiers in Bioscience Scholar

Front. Biosci. (Schol Ed) **2025**; 17(2): 33398
https://doi.org/10.31083/FBS33398

Original Research

Staphylococcus aureus Strains With a Negative Coagulase Tube Test are Associated With Staphylocoagulase Genotypes

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Academic Editor: Anna Aiello
Submitted: 29 November 2024 Revised: 5 February 2025 Accepted: 26 February 2025 Published: 16 June 2025

Abstract

Background: *Staphylococcus aureus* is a significant human pathogen. Therefore, differentiating *Staphylococcus aureus* (*S. aureus*) from coagulase-negative staphylococcal species is an important step in the diagnostics procedure. The coagulase tube test assay is used as a preliminary identification test; however, there are instances of *S. aureus* isolates testing negative. We hypothesized that this might affect clinical outcomes and that particular staphylocoagulase genotypes are not detected by the coagulase tube test. **Methods**: In total, 122 clinical bloodstream *S. aureus* isolates with clinical metadata were examined for coagulating ability. The *coa* genotype was determined for each isolate using whole genome sequencing, and regions flanking the *coa* gene in the genome sequence were examined for synteny to identify differences that may indicate possible differences in *coa* gene regulation. In addition, a subset of isolates was assessed for *coa* gene expression using reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR). **Results**: All 122 isolates were found to have the *coa* gene, and all but one tested positive in the coagulase slide test. Comparatively, 18.9% of the isolates tested negative in the coagulase tube test assay. There was no association between an isolate having a negative tube test and having a complicated bloodstream infection. Among the 122 isolates, 11 *coa* genotypes were present, with similarities between the *coa* gene and comparative genome phylogenies and grouping of multilocus sequence types (MLST, abbreviated to ST), indicating that the *coa* gene may be vertically inherited. Staphylocoagulase type X and XI isolates were more likely to test negative in the coagulase tube test despite evidence of an intact functional *coa* gene. **Conclusions**: The *S. aureus* lineages may be negative in the coagulase tube test, especially ST15 and ST3911 (from staphylocoagulase genotype X). Our analysis suggests that the observed negativity in the coagulase tube test is due to the inability of particular coagulase types to coagulate the substrate provided in the commercial test. This has implications for using the tube test in differentiating *Staphylococcus aureus* isolates from other species. The Illumina genome sequencing read-set for each isolate was submitted to the National Center for Biotechnology Information (NCBI) under the accession number PRJNA611667.

Keywords: *Staphylococcus aureus*; diagnostic errors; bacteremia; microbiological techniques

1. Introduction

Accurate species identification of a bacterial isolate is critical for managing infection in hospitalized patients. *Staphylococcus aureus* (*S. aureus*) is an important species to identify and is traditionally differentiated from coagulase-negative staphylococcal species through the coagulase tube test. *S. aureus* is frequently associated with complications and was responsible for over one million deaths globally in 2019 [1]. Conversely, most coagulase-negative staphylococcal species are rarely associated with disease; thus, coagulase testing of staphylococcal isolates is central to patient management.

S. aureus has multiple proteins that interact with and target platelets and host hemostasis pathways. Some examples include the clumping factors, which bind to fibrinogen or fibrin to create large cellular clumps [2]. Another significant protein is staphylocoagulase, which activates the clotting cascade by binding to complement C3, activating prothrombin to thrombin [3].

Diagnostically, this coagulating ability can be detected 4–24 hours after collection and well ahead of formal species identification using molecular methods. Developed in 1940, Fairbrother identified that combining an *S. aureus* colony with plasma in a tube would cause coagulation, later described as staphylocoagulase activity [4]. Subsequently, it was found that substituting rabbit plasma for human plasma would reduce the coagulase testing time [5], making it a staple phenotypic assay in the diagnostics lab for infections.

Advancements in understanding the underlying genetic makeup of *S. aureus* and other bacterial species have increased, allowing for high-throughput systems that can use unique genetic and biochemical markers to identify bacterial species quickly; however, most systems still require culturing first [6].

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Some advanced diagnostic tests include molecular-based platforms such as the Xpert MRSA/SA BC assay and FilmArray systems, which use genetic probes and polymerase chain reactions [6]. One key advancement was the mass spectroscopy techniques, such as the gold-standard matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), which increased the accuracy and reliability of the identification of species and drug resistance markers over phenotypic tests, with comparable times to phenotypic tests such as the coagulase tube test [6]. MALDI-TOF MS does have some diagnostic limitations, although not with *S. aureus*. Indeed, MALDI-TOF MS is known for its expense, meaning it is not a primary diagnostic tool for the laboratory [7]. Therefore, some laboratories still use the coagulase tube test as either a primary or presumptive identification; however, significantly, some *S. aureus* isolates have been observed to test negative [8,9].

The reported rate of coagulase-negative *S. aureus* isolates ranges between 2% and 16% [9–11]. Meanwhile, the reasons for this range remain unclear, with one study suggesting that the high rate of negative isolates may be due to the high number of antibiotic-resistance genes present in an isolate [12]. Another possibility is that upstream and downstream regulations prevent the *staphylocoagulase* gene from being expressed. Prior studies have identified that the accessory gene regulator (Agr) regulates *staphylocoagulase* [13,14], meaning Agr mutant strains could potentially affect coagulase expression. However, to our knowledge, a study has not focused on the genetic expression of this gene since the late 1990s, with more recent studies focusing on coagulation in coagulase-negative *staphylococci* species [15] or effects on production with novel chemical compounds [16,17].

A potential reason for coagulase-negative isolates is the variation in specificity of the *staphylocoagulase* gene (*coa*). In the late 1990s, classification of *staphylocoagulase* types via serotyping experiments began [18]. Through the early 2000s, the serotyping scheme was correlated with the *coa* genotype [18]. The genotyping scheme has been extended from an initial 10 serotypes to 16 genotypes of *Staphylococcus aureus* complexes, including several subtypes, such as *Staphylococcus argenteus* [19,20].

The staphylocoagulase protein contains six recognized regions: the C-terminal, D1, D2, central and tandem repeat regions, and N-terminal regions [18]. The genotype is determined from the nucleotide sequence encoding the D1, D2, and central regions. The C-terminal and D1 regions bind to fibrinogen, and the N-terminal regions play a role in prothrombin activation, suggesting that the D1 variability may play a role in coagulase binding specificity, which is important in the coagulase tube test [18]. Interestingly, to our knowledge, no expression studies have focused on staphylocoagulase expression at the nucleic level, with most studies using Western blot assays; no studies outside of functional characterization have assessed how these variations affect expression and, therefore, how it may impact diagnostics.

This study aimed to investigate the reasons for *Staphylococcus aureus* isolates testing negative in the coagulase tube test and its implications on healthcare. We aimed to identify whether there was an association between *coa* genotypes and negative results in the coagulase tube test. We also aimed to assess if there were potential factors outside of the *coa* genotype that could explain this negative phenotype. Finally, by investigating these factors, we aimed to determine the relevance of the coagulase tube test in a molecular testing era, which will improve the diagnostic and clinical decision-making ability of this deadly infection.

2. Materials and Methods

2.1 Isolates

This study used 122 *S. aureus* clinical isolates collected from adult human bloodstream infections in the Barwon South-West region of Victoria, Australia. A previous study defined clinical metadata, such as complications associated with these isolates [21].

2.2 Coagulase Production Detection

The 122 isolates were assessed using the coagulase slide and tube test per the manufacturer's instructions (Remel, Thermo Fisher Scientific, Scoresby, Australia) [22]. All isolates were evaluated in biological triplicate. Isolates that tested negative for coagulase production were assessed twice to confirm test results. The *S. aureus* strain ATCC®35556 was used as the positive control.

2.3 Accessory Gene Regulator Function

Accessory gene regulator function was assessed using the Christie–Atkins–Munch-Petersen (CAMP) assay [23]. Briefly, this consisted of streaking a line of the sample perpendicular to a disc of purified beta-hemolysin on blood agar (Thermo Fisher Scientific, Scoresby, Australia). Following 24 hours of incubation, a zone of enhanced hemolysis indicates positive accessory gene regulator function.

2.4 Assessing the Staphylocoagulase Genotypes of Clinical S. aureus Isolates

The genomes of each isolate were sequenced at the Peter Doherty Institute, University of Melbourne, Australia, using previously described methods [24]. Briefly, isolate genome DNA was extracted using the Qiagen DNeasy Blood and Tissue Extraction kits (Qiagen, Clayton, Australia). Illumina sequencing libraries were prepared from the genomic DNA using a shotgun sequencing strategy, and then libraries were run on the Illumina NextSeq 500 instrument (Illumina, Melbourne, Australia) (paired-end, 150 base reads).

The Nullarbor pipeline (V2.0.20181015) [25] was used to perform the assembly and characterization of the genome sequence of each isolate; the software used included SPAdes (V3.13.0) [26] (genome assembly), PROKKA (V1.13.3) [27] (genome annotation), MLST (V2.16.1) [28–30] (sequence typing), and Kraken (V1.0) [31] (taxonomic classification and isolate purity check). The annotated genome sequence produced by PROKKA was used to identify the *staphylocoagulase* (*coa*) gene. Visualizations of the regions upstream and downstream of the *coa* gene were performed using Artemis (V18.2.0) [32]. Read-sets for each isolate were submitted to the National Center for Biotechnology Information (NCBI) under the accession number PRJNA611667, and a summary of bioinformatic data is available in **Supplementary Table 1**. The *staphylocoagulase* sequence was extracted and compared to the reference sequences of each staphylocoagulase genotype using BLAST; the genotype reference sequences were found in Watanabe *et al*. [33].

2.5 Coagulase Expression

A subset of isolates was assessed in technical triplicates using reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) analysis of *coa* gene expression. Isolates were chosen to represent a genotype or from coagulase test results to include both positive and negative isolates.

RT-qPCR was performed as follows: Overnight cultures of the isolates were diluted to 0.1 optical density ($OD_{600\ nm}$) in fresh medium and then grown for 3 hours. The culture conditions are known to result in *coa* gene expression [13]. Cultures were pelleted and treated with Qiagen RNAProtect (Qiagen, Clayton, Vic, Australia). to preserve the RNA. Preserved cell suspensions were extracted using the Qiagen RNA Bacterial Mini kit (Qiagen, Clayton, Australia). as previously described [34], including the pretreatment of the cell suspensions with staphylolysin (Sigma Aldrich, Merck, Bayswater, Australia) [35]. CDNA was synthesized using the Superscript IV VILO kit (Invitrogen, Thermo Fisher Scientific, Scoresby, Australia). Real-time PCR was performed using Applied Biosystems *Power* Sybr master mix kit (Thermo Fisher Scientific, Scoresby, Australia) on the QuantStudio 6 Flex (Version 1.1.2 Applied Biosystems, Thermo Fisher Scientific, Scoresby, Australia) with cycling conditions of 95 °C for 10 min, 40 cycle stage (95 °C for 15 s, 60 °C for 1 min, a dissociation step at 95 °C for 15 s) and finishing as 60 °C for 1 min. The primers used for this study are listed in Table 1 (The *coa* gene primers were designed in this study, and GyraseB (*gyrB*) primers (a housekeeping gene for use as a control) were obtained from a prior study [35] and manufactured by Integrated DNA technologies (Melbourne, Australia)).

The DNase-treated RNA samples were checked for residual DNA. The residual DNA was measured and assessed against the cDNA real-time results using the paired Wilcoxon rank test to ensure it was not attributed to gene expression. These results are graphically represented in **Supplementary Fig. 1**.

Table 1. Primers used in this study.

Primer	Sequence
Coa-F	GGT CCG AGA CCG CAA TTT A
Coa-R	ATC TTG GTC TCG CTT CAT ATC C
GyrB-F	CCA GGT AAA TTA GCC GAT TGC
GyrB-R	AAA TCG CCT GCG TTC TAG AG

Coa, Staphylocoagulase; *GyrB*, GyraseB.

The results are reported with expressions noted as plus or minus, denoting positive or negative expressions. The raw CT measurements are recorded in **Supplementary Table 2**.

2.6 Statistical Analysis

The results of the coagulase tube test were compared within genotypes of 10 or more representative isolates. Coagulase results were assessed against clinical outcomes. Data are presented as percentages (numbers), and logistic regression data are presented as odds ratios with 95% confidence intervals. Exploratory regression analysis was performed using SPSS V29.0 (IBM Corp., Armonk, NY, USA), and GraphPad Prism V9.0 (Dotmatics, Boston, MA, USA) was used to calculate the paired Wilcoxon rank test and create graphical figures.

3. Results

Of the 122 isolates used in this study, 81.1% (n = 99) of the cohort were positive for coagulase function via the tube test. All but one isolate was positive via the coagulase slide test. The negative coagulase phenotypes were not observed to be significantly associated with complications in bacteremia (**Supplementary Table 3**).

All isolates were confirmed as *S. aureus* via sequencing as evaluated using Kraken2 and on assembled genome size. A summary of the sequencing data can be found in **Supplementary Table 1**. All 122 isolates were found to have the *staphylocoagulase* gene. However, 20% of the isolates had the *coa* gene across two contigs. The staphylocoagulase genotype I-XI, but not genotype IX, was detected in the isolate collection, as shown in Table 2. Genotype II was most frequently identified (n = 33), followed by genotype IV (n = 26). Where multiple isolates had the multilocus sequence types (MLSTs), that subset of isolates had the same *coa* genotype.

By comparing coagulase tube test results with *coa* genotype isolates with genotypes VII, X, and XI, it was observed that these results were more likely to test negative for the tube test. These results are summarized in **Supplementary Table 3**. Due to the small sample size, an exploratory univariable regression model for types II, X, and VII was assessed statistically as these types had indicated negative isolates and had 10 or more representative isolates. Staphylocoagulase type X (OR: 5.22, 95% confidence interval (CI):

Table 2. A summary of the distribution of *coa* genotype, sequence type, and coagulase test result of the cohort.

Genotype	Geno subtypes	Number of isolates	Sequence type (ST)	Coagulase positive production, n (%)
I	Ib	1	ST672	1/1 (100)
II	IIa, IIb	33	ST5, ST25, ST6315*	27/33 (81.8)
III	IIIa	8	ST7, ST8 ST88, ST630	7/8 (87.5)
IV	IVa, IVb	22	ST6, ST30, ST34, ST39, ST1083, ST6314*, ST6317*	26/26 (100)
V	Va, Vb	10	ST72, ST121, ST188	9/10 (90)
VI	VIc	4	ST25, ST97, ST953	4/4 (100)
VII	VIIa, VIIb, VIIc	22	ST1, ST45, ST59, ST156, ST291, ST508, ST398, ST6316*, ST6320*	15/22 (68.2)
VIII	VIIIb	1	ST6319*	1/1 (100)
X	Xa	10	ST15, ST3911	5/10 (50)
XI	XIa	7	ST22, ST737, ST3197, ST6318*	4/7 (57.1)

*Novel sequence type identified in this collection of isolates; ST, sequence type.

[1.37, 19.91], p = 0.015) was statistically associated with a negative tube test. Staphylocoagulase type VII was not statistically significant (OR: 2.45, 95% CI: [0.86, 6.96], p = 0.093).

To assess for potential contributing factors outside of the genotype, we evaluated the structure of the gene and the surrounding genes upstream and downstream of the *coa* gene in each isolate. Only one isolate sequence had a large deletion at the 3′ end of the gene, which might theoretically affect staphylocoagulase expression and function. The phenotypic results concurred with this inference from the gene synteny analysis. This isolate was classified as genotype XI. Within genotypes, there were slight SNP differences within the variant regions; however, no SNP differences were consistently observed with the negative tube test results.

The majority of isolates had a common gene order close to the *coa* gene, consisting of carobamoyl-phosphate synthase small chain (*carA*), long chain fatty acid CoA ligase (*lcfb*), crotonobetainyl-CoA reductase (*caiA*), 3-hydroxyacyl-CoA dehydrogenase (*fadN*), probable acetyl-Coa acyltransferase (*fadA*), upstream, and Staphylococcal complement inhibitor (*scn*), a hypothetical gene, pyruvate formate lyase activating enzyme (*pflA*), formate acetyltransferase (*pflB*), and a hypothetical protein (unknown function) downstream. Slight differences observed in the upstream and downstream genes did not correlate with the coagulase tube test result and did not differ by genotype.

As Agr is known to affect coagulase production indirectly, we assessed Agr function to determine if poor Agr function correlated with a negative coagulase tube test; however, no association was observed (OR: 0.50, 95% CI: [0.14, 1.82], p = 0.290; **Supplementary Table 3**).

With genetic structure and regulatory function not accounting for all the negative tube tests observed, we selected a subset of isolates representing different genotypes and coagulase tube test results to assess whether the *coa* gene is being expressed via reverse transcriptase real-time PCR. The isolates tested for *coa* gene expression are shown in Table 3. The tested isolates included positive coagulase tube test isolates from genotypes II, IV, and X and negative tube tests from genotypes II, VII, and X, with two isolates representing negative X isolates.

RNA was extracted from cultures grown under conditions for *coa* gene expression [13]. Expression of *coa* was detected in all tested isolates despite the negative coagulase tube test observed in some of them (see **Supplementary Table 3** for individual results). One isolate (isolate 27, the type IV, coagulase tube test positive) had very low *coa* expression under these growth conditions (see **Supplementary Table 2** for details).

4. Discussion

The severity of disease associated with *S. aureus* infections highlights the importance of rapid and accurate identification of this organism in the laboratory. The coagulase tube test has been used since the 1940s to identify or presumptively identify *S. aureus*. The test is still used in resource-limited countries and presumptively even in countries with access to MALDI-TOF for isolate identification [36]. However, there are reports of *S. aureus* isolates that test negative in the coagulase tube test [37]. Among the 122 *S. aureus* isolates in this study, there was a tube test negative rate of 19.8%, similar to that seen in several prior studies [9–11,38].

In this study, negative coagulase tube test results were not associated with complications in bacteremia. However, there was no significant negative association, suggesting that isolates that test negative in the coagulase tube test are no less virulent than those that do. Therefore, we investigated why some *S. aureus* isolates test negative in the coagulase tube test.

At the genetic level, all isolates possessed the *staphylocoagulase* gene. Only one isolate (isolate 116) had a major deletion in the conserved region of the *coa* gene that may affect expressed protein function (that could have theoretically affected coagulase tube test positivity) and was observed to be coagulase tube test negative. There were also

Table 3. Isolates assessed for *coa* gene expression.

Isolate ID	Staphylocoagulase genotype	Sequence type (ST)	Coagulase tube test	Agr function	*coa* expression
26	X	ST15	+	+	+
27	IV	ST30	+	+	+*
28	II	ST5	-	+	+
95	VII	ST45	-	+	+
105	X	ST3911	-	+	+
30	II	ST5	+	+	+
32	X	ST15	-	+	+

*Only one of three technical replicates indicated expression and only at cycle 38.44; + indicates a positive result; - indicates no detectable function; Agr, accessory gene regulator.

no differences in the upstream and downstream genes from the *staphylocoagulase* gene that were exclusively found in tube test-negative isolates that may have affected *coa* gene expression in the isolates. In total, 20% of the isolates had the *coa* gene split across two contigs due to the tandem repeat structure of the gene, a known effect in short-read sequencing technology where repeat sequences are not adequately spanned by reads [39]. Despite the *coa* gene being split across two contigs in the draft genome sequences of those isolates, we could identify all *coa* gene regions and the contig breakpoint at the tandem repeat. Indeed, due to this limitation, repeat sequence elements in the genome often confound the reproduction of other typing methods, such as the pulse field electrogram fragment lengths [40]. In detecting the *coa* gene, genotyping and the analysis presented in this study were not affected by the poor assembly across the tandem repeat regions. Meanwhile, long-read sequencing would eliminate this assembly anomaly.

The Agr function indirectly affects coagulase production [13] and was not associated with negative coagulase tube test results. Other gene expression regulators may be related to the tube test result; however, given the association between genotype and tube test result, no additional regulators were investigated.

We next investigated the genetic variation in the *staphylocoagulase* gene. First identified molecularly by Watanabe *et al*. [33], the *staphylocoagulase* gene has different genotypes (identified initially by serotyping tests). The cohort isolate genes were mapped against references, and it was identified that all staphylocoagulase genotypes I–XII except type IX were present. This was not unexpected, as genotype IX is found in animal-associated isolates [41]. The staphylocoagulase genotypes are lineage-associated, and the gene is likely to be vertically inherited in *S. aureus* [33]. This is consistent with the observation from our isolates, whereby all isolates with the same ST have the same *coa* genotype.

Significantly, staphylocoagulase genotype X was negatively associated with a positive coagulase tube test result (OR: 5.22, 95% CI: [1.37, 19.10], $p = 0.015$). Genotype X was exclusively found in ST15 and ST3911, both of which are a part of clonal complex 15. This concurred with a prior study that found type X, sub-type Xa, in ST15 strains [33], although the preceding study only had one representative isolate.

In relation to the collection of isolates, variation in *S. aureus* ST prevalence across geographical areas has been observed previously. For example, the most common *coa* genotype observed was type II, usually ST5 and ST25; ST5 is the most common in Australia [42,43]. The association between the *coa* genotype and ST types may explain the rates of coagulase-negative *S. aureus* isolates. ST15 is a common strain globally, but isolation rates differ depending on geographical region [44,45]. The observation that ST15 may be more likely to be tube test negative could also explain the published variations in coagulase-negative *S. aureus* isolates.

We investigated whether there were other reasons preventing expression. Using real-time reverse transcriptase PCR, we observed that all isolates in the selected subset expressed staphylocoagulase. In cases where the isolates were coagulase-negative by tube test, this shows that staphylocoagulase is likely to be produced; however, this coagulase activity is not detected, perhaps due to possible variations in coagulase substrate specificity for particular genotypes.

S. aureus and mammals have co-existed and coevolved for millennia [46]. Hence, coevolution with a particular animal host might narrow the staphylocoagulase substrate; for example, coagulase type IX is exclusively found in livestock-associated strains, so it may be that the rabbit plasma used in the coagulase test is not a suitable substrate for *coa* genotype X. Interestingly, a 2010 study investigated isolates collected from human, bovine, and dog plasma, and identified that dog plasma was superior to rabbit plasma. Although there were no coagulase-negative isolates, it should be noted that the collection of isolates was determined using a coagulate test [47].

An alternative driver for the postulated shift in specificity is that there may have been a functional shift in the affected genotypes. Staphylocoagulase type X has 98.5% nucleotide identity similarity with the *fibronectin-binding protein A* gene [11], so a change in function may impact the coagulation normally observed in the tube test.

With fast molecular techniques increasingly used in the clinical setting and the coagulase test used only as a preliminary test, whether it is time to move on from coagulase production as a marker for *S. aureus* should be posited. Numerous rapid molecular techniques have been developed recently, including the MALDI-TOF and BinaxNow with BinaxNow PBP2a assay [6]. While some methods, such as MALDI-TOF, have become routine in clinical laboratories, issues with these rapid diagnostics are limited to low throughput or high costs [6]. Therefore, avoiding the coagulase tube test may not be possible as it is cheap and has high throughput, making it an efficient preliminary test. However, this work highlights the importance of being aware of the limitations of the coagulase tube test.

This study has limitations, predominantly the small sample size, which could potentially make the regression association more prone to type II errors. Therefore, to minimize these errors, we only statistically assessed the samples with representative samples of 10 or more isolates and those with a distribution under 90%. Future studies investigating this ST would be beneficial in confirming this study's findings.

Another limitation is the study setting: Study isolates were collected from a single-site collection point, meaning the genetic diversity represented in this study may not accurately reflect other clinical settings. The isolates were also all clinical, meaning the lineages were not evenly distributed, and the contribution of clonal complex 15 strains was small. However, this reflects the community-associated bloodstream lineages and incidence rates observed in a clinical laboratory setting for this site.

5. Conclusions

Despite these limitations, to our knowledge, this study is the first to identify associations between the staphylocoagulase genotype and coagulase tube test results, the first to use RT-PCR to determine whether coagulase-negative strains of *S. aureus* express the *staphylocoagulase* gene, and the first to see if Agr function would affect the coagulase tube test.

To conclude, this study does not recommend routinely screening for the *staphylocoagulase* gene for clinical or diagnostic purposes or removing the coagulase tube test from clinical practice. However, it does provide an important reminder that these bacterial lineages may have discrepancies in traditional laboratory tests. We observed that specific lineages, including those from MLST15 and MLST3911, may be more likely to test negative in a coagulase tube test, and scientists should be aware of false negatives.

Availability of Data and Materials

The readsets for each isolate have been submitted to NCBI under the accession number PRJNA611667. All data not shown in the supplementary data and reported on in this paper will also be shared by the corresponding author upon reasonable request.

Author Contributions

CB and EA designed the research study. CB performed the research. EA provided help and advice on the clinical significance of the findings. CB and DB analysed the data. CB and DB wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors have read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was carried out in accordance with the guidelines of the Declaration of Helsinki. Ethical approval for this project was granted by the Barwon Health HREC (Reference number 16/33) and acknowledged by the Deakin University HREC (Reference number 2017-217). Due to the negligible to low risk and the retrospective data collection of this study, the Barwon Health HREC granted a waiver of consent for this study.

Acknowledgment

We gratefully acknowledge the assistance and instruction from Dr Glen Carter and Professor Ben Howden of the Peter Doherty Institute for sequencing.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBS33398.

References

[1] GBD 2019 Antimicrobial Resistance Collaborators. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet (London, England). 2022; 400: 2221–2248. https://doi.org/10.1016/S0140-6736(22)02185-7.

[2] Herman-Bausier P, Labate C, Towell AM, Derclaye S, Geoghegan JA, Dufrêne YF. *Staphylococcus aureus* clumping factor A is a force-sensitive molecular switch that activates bacterial adhesion. Proceedings of the National Academy of Sciences of the United States of America. 2018; 115: 5564–5569. https://doi.org/10.1073/pnas.1718104115.

[3] Malachowa N, Kobayashi SD, Porter AR, Braughton KR, Scott DP, Gardner DJ, *et al*. Contribution of Staphylococcus aureus Coagulases and Clumping Factor A to Abscess Formation in a Rabbit Model of Skin and Soft Tissue Infection. PloS One. 2016; 11: e0158293. https://doi.org/10.1371/journal.pone.0158293.

[4] Fairbrother RW. Coagulase production as a criterion for the classification of the staphylococci. The Journal of Pathology and Bacteriology. 1940; 50: 83–88. https://doi.org/10.1002/path.1700500112.

[5] Chapman GH, Berens C, Stiles MH. The Coagulation of Plasma by Staphylococci. Journal of Bacteriology. 1941; 41: 431–440. https://doi.org/10.1128/jb.41.4.431-440.1941.

[6] Duan R, Wang P. Rapid and Simple Approaches for Diagnosis of *Staphylococcus aureus* in Bloodstream Infections. Polish Journal of Microbiology. 2022; 71: 481–489. https://doi.org/10.33073/pjm-2022-050.

[7] Elbehiry A, Aldubaib M, Abalkhail A, Marzouk E, ALbeloushi A, Moussa I, *et al*. How MALDI-TOF Mass Spectrometry Technology Contributes to Microbial Infection Control in Healthcare Settings. Vaccines. 2022; 10: 1881. https://doi.org/10.3390/vaccines10111881.

[8] Woo PC, Leung AS, Leung KW, Yuen KY. Identification of slide coagulase positive, tube coagulase negative Staphylococcus aureus by 16S ribosomal RNA gene sequencing. Molecular Pathology: MP. 2001; 54: 244–247. https://doi.org/10.1136/mp.54.4.244.

[9] Pourshadi M, Klaas J. Evaluation of latex agglutination and microtube coagulase tests for detection of Staphylococcus aureus. Diagnostic Microbiology and Infectious Disease. 1984; 2: 287–291. https://doi.org/10.1016/0732-8893(84)90059-2.

[10] Kateete DP, Kimani CN, Katabazi FA, Okeng A, Okee MS, Nanteza A, *et al*. Identification of Staphylococcus aureus: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. Annals of Clinical Microbiology and Antimicrobials. 2010; 9: 23. https://doi.org/10.1186/1476-0711-9-23.

[11] Shahmohammadi S, Sheikh AF, Shahin M, Mir I. Evaluation of Antibiotic Susceptibility Pattern and Distribution of coa Genes in Coagulase-negative S. aureus from Ahvaz, Iran. Infectious Disorders Drug Targets. 2019; 19: 383–387. https://doi.org/10.2174/1871526518666180816101629.

[12] Młynarczyk G, Kochman M, Lawrynowicz M, Fordymacki P, Młynarczyk A, Jeljaszewicz J. Coagulase-negative variants of methicillin-resistant Staphylococcus aureus subsp. aureus strains isolated from hospital specimens. Zentralblatt Fur Bakteriologie: International Journal of Medical Microbiology. 1998; 288: 373–381. https://doi.org/10.1016/s0934-8840(98)80010-8.

[13] Lebeau C, Vandenesch F, Greenland T, Novick RP, Etienne J. Coagulase expression in Staphylococcus aureus is positively and negatively modulated by an agr-dependent mechanism. Journal of Bacteriology. 1994; 176: 5534–5536. https://doi.org/10.1128/jb.176.17.5534-5536.1994.

[14] Phonimdaeng P, O'Reilly M, O'Toole PW, Foster TJ. Molecular cloning and expression of the coagulase gene of Staphylococcus aureus 8325-4. Journal of General Microbiology. 1988; 134: 75–83. https://doi.org/10.1099/00221287-134-1-75.

[15] Pickering AC, Yebra G, Gong X, Goncheva MI, Wee BA, MacFadyen AC, *et al*. Evolutionary and Functional Analysis of Coagulase Positivity among the Staphylococci. MSphere. 2021; 6: e0038121. https://doi.org/10.1128/mSphere.00381-21.

[16] Ge B, Hu C, Qian Y, Tang Y, Zhang Q, Jiang S, *et al*. Sinensetin interferes with Staphylococcus aureus infections by targeting staphylocoagulase and improves infection survival rates in mouse model of pneumonia. Journal of Applied Microbiology. 2024; 135: lxae235. https://doi.org/10.1093/jambio/lxae235.

[17] Zhang C, Zhang W, Zhu S, Hu C, Che S, Wang M, *et al*. Bilobetin attenuates Staphylococcus aureus virulence by targeting Von Willebrand factor-binding protein and staphylocoagulase. World Journal of Microbiology & Biotechnology. 2023; 39: 358. https://doi.org/10.1007/s11274-023-03812-z.

[18] Watanabe S, Ito T, Takeuchi F, Endo M, Okuno E, Hiramatsu K. Structural comparison of ten serotypes of staphylocoagulases in Staphylococcus aureus. Journal of Bacteriology. 2005; 187: 3698–3707. https://doi.org/10.1128/JB.187.11.3698-3707.2005.

[19] Kyaw WK, Aung MS, San T, Maw WW, Mu KK, Mon WLY, *et al*. Molecular Epidemiological Characterization of *Staphylococcus aureus* and *Staphylococcus argenteus* Clinical Isolates from a National Tertiary Care Hospital in Myanmar: Co-Isolation of Multiple Clones and Identification of Novel Staphylocoagulase Genotype. Microbial Drug Resistance (Larchmont, N.Y.). 2023; 29: 127–137. https://doi.org/10.1089/mdr.2022.0191.

[20] Aung MS, Urushibara N, Kawaguchiya M, Hirose M, Ike M, Ito M, *et al*. Distribution of Virulence Factors and Resistance Determinants in Three Genotypes of *Staphylococcus argenteus* Clinical Isolates in Japan. Pathogens (Basel, Switzerland). 2021; 10: 163. https://doi.org/10.3390/pathogens10020163.

[21] Botheras CL, Bowe SJ, Cowan R, Athan E. C-reactive protein predicts complications in community-associated S. aureus bacteraemia: a cohort study. BMC Infectious Diseases. 2021; 21: 312. https://doi.org/10.1186/s12879-021-05962-7.

[22] Remel. Coagulase Plasma Manual (Rabbit Plasma w/EDTA). 2022. Available at: https://www.thermofisher.com/order/catalog/product/R21060 (Accessed: 5 May 2023).

[23] Laabei M, Jamieson WD, Massey RC, Jenkins ATA. Staphylococcus aureus interaction with phospholipid vesicles–a new method to accurately determine accessory gene regulator (agr) activity. PloS One. 2014; 9: e87270. https://doi.org/10.1371/journal.pone.0087270.

[24] Baines SL, Howden BP, Heffernan H, Stinear TP, Carter GP, Seemann T, *et al*. Rapid Emergence and Evolution of Staphylococcus aureus Clones Harboring fusC-Containing Staphylococcal Cassette Chromosome Elements. Antimicrobial Agents and Chemotherapy. 2016; 60: 2359–2365. https://doi.org/10.1128/aac.03020-15.

[25] Seemann T, Goncalves da Silva A, Bulach DM, Schultz MB, Kwong JC, Howden BP. Nullarbor. Github [Internet]. Available at: https://github.com/tseemann/nullarbor (Accessed: 4 April 2023).

[26] Prjibelski A, Antipov D, Meleshko D, Lapidus A, Korobeynikov A. Using SPAdes De Novo Assembler. Current Protocols in Bioinformatics. 2020; 70: e102. https://doi.org/10.1002/cpbi.102.

[27] Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics (Oxford, England). 2014; 30: 2068–2069. https://doi.org/10.1093/bioinformatics/btu153.

[28] Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. Journal of Clinical Microbiology. 2000; 38: 1008–1015. https://doi.org/10.1128/JCM.38.3.1008-1015.2000.

[29] Seemann T. MLST. 2022. Available at: https://github.com/tseemann/mlst (Accessed: 4 April 2023).

[30] Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Research. 2018; 3: 124. https://doi.org/10.12688/wellcomeopenres.14826.1.

[31] Wood DE, Salzberg SL: Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biology 2014, 15: R46. https://doi.org/10.1186/gb-2014-15-3-r46.

[32] Carver T, Harris SR, Berriman M, Parkhill J, McQuillan JA. Artemis: an integrated platform for visualization and analysis of high-throughput sequence-based experimental data. Bioinformatics (Oxford, England). 2012; 28: 464–469. https://doi.org/10.1093/bioinformatics/btr703.

[33] Watanabe S, Ito T, Sasaki T, Li S, Uchiyama I, Kishii K, *et al*. Genetic diversity of staphylocoagulase genes (coa): insight

into the evolution of variable chromosomal virulence factors in Staphylococcus aureus. PloS One. 2009; 4: e5714. https://doi.org/10.1371/journal.pone.0005714.
[34] Baines SL, Holt KE, Schultz MB, Seemann T, Howden BO, Jensen SO, *et al*. Convergent adaptation in the dominant global hospital clone ST239 of methicillin-resistant Staphylococcus aureus. mBio. 2015; 6: e00080. https://doi.org/10.1128/mBio.00080-15.
[35] Gomes-Fernandes M, Laabei M, Pagan N, Hidalgo J, Molinos S, Villar Hernandez R, *et al*. Accessory gene regulator (Agr) functionality in Staphylococcus aureus derived from lower respiratory tract infections. PloS One. 2017; 12: e0175552. https://doi.org/10.1371/journal.pone.0175552.
[36] Singhal N, Kumar M, Kanaujia PK, Virdi JS. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Frontiers in Microbiology. 2015; 6: 791. https://doi.org/10.3389/fmicb.2015.00791.
[37] Mackay AD, Quick A, Gillespie SH, Kibbler CC. Coagulase-negative methicillin-resistant Staphylococcus aureus infection. Lancet (London, England). 1993; 342: 492. https://doi.org/10.1016/0140-6736(93)91615-s.
[38] Vandenesch F, Bes M, Lebeau C, Greenland T, Brun Y, Etienne J. Coagulase-negative Staphylococcus aureus. Lancet (London, England). 1993; 342: 995–996. https://doi.org/10.1016/0140-6736(93)92045-u.
[39] Tørresen OK, Star B, Mier P, Andrade-Navarro MA, Bateman A, Jarnot P, *et al*. Tandem repeats lead to sequence assembly errors and impose multi-level challenges for genome and protein databases. Nucleic Acids Research. 2019; 47: 10994–11006. https://doi.org/10.1093/nar/gkz841.
[40] Dallal MMS, Khoramizadeh MR, Amiri SA, Yaraghi AAS, Fard RMN. Coagulase gene polymorphism of Staphylococcus aureus isolates: A study on dairy food products and other foods in Tehran, Iran. Food Science and Human Wellness. 2016; 5: 186–190. https://doi.org/10.1016/j.fshw.2016.09.004.
[41] Kinoshita M, Kobayashi N, Nagashima S, Ishino M, Otokozawa S, Mise K, *et al*. Diversity of staphylocoagulase and identification of novel variants of staphylocoagulase gene in Staphylococcus aureus. Microbiology and Immunology. 2008; 52: 334–348. https://doi.org/10.1111/j.1348-0421.2008.00045.x.
[42] Coombs GW, Yee NWT, Daley D, Bennett CM, Robinson JO, Stegger M, *et al*. Molecular Epidemiology of Penicillin-Susceptible *Staphylococcus aureus* Bacteremia in Australia and Reliability of Diagnostic Phenotypic Susceptibility Methods to Detect Penicillin Susceptibility. Microorganisms. 2022; 10: 1650. https://doi.org/10.3390/microorganisms10081650.
[43] Marshall HS, Baber J, Richmond P, Nissen M, Shakib S, Kreiswirth BN, *et al*. S. aureus colonization in healthy Australian adults receiving an investigational S. aureus 3-antigen vaccine. The Journal of Infection. 2019; 79: 582–592. https://doi.org/10.1016/j.jinf.2019.09.018.
[44] Ibrahim RA, Mekuria Z, Wang SH, Mediavilla JR, Kreiswirth B, Seyoum ET, *et al*. Clonal diversity of Staphylococcus aureus isolates in clinical specimens from selected health facilities in Ethiopia. BMC Infectious Diseases. 2023; 23: 399. https://doi.org/10.1186/s12879-023-08380-z.
[45] Holtfreter S, Grumann D, Balau V, Barwich A, Kolata J, Goehler A, *et al*. Molecular Epidemiology of Staphylococcus aureus in the General Population in Northeast Germany: Results of the Study of Health in Pomerania (SHIP-TREND-0). Journal of Clinical Microbiology. 2016; 54: 2774–2785. https://doi.org/10.1128/JCM.00312-16.
[46] Wong Fok Lung T, Chan LC, Prince A, Yeaman MR, Archer NK, Aman MJ, *et al*. *Staphylococcus aureus* adaptive evolution: Recent insights on how immune evasion, immunometabolic subversion and host genetics impact vaccine development. Frontiers in Cellular and Infection Microbiology. 2022; 12: 1060810. https://doi.org/10.3389/fcimb.2022.1060810.
[47] Rubin JE, Bayly MK, Chirino-Trejo M. Comparison of dog and rabbit plasmas in the tube coagulase test for Staphylococcus aureus. Journal of Veterinary Diagnostic Investigation: Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc. 2010; 22: 770–771. https://doi.org/10.1177/104063871002200521.